Upsala J Med Sci 91: 143–146, 1986

# The Kodak Ektachem Dry Layer Technology for Clinical Chemistry

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The manufacture of Photographic film requires a very high precision coating technology with the ability to layer emulsions very uniformly onto supports and underlying layers. We asked ourselves were there other areas where Kodak's ability to coat very thin chemical layers could be used? Research showed that this Technology could be applied to Clinical Chemistry. The big problem to overcome was to get enzymes to react normally after being thin layer coated onto film. Once this problem had been overcome, the EKTACHEM Programme was born.

The EKTACHEM Dry Layer Technology can be applied to Clinical Chemistry for both Colorimetric and Potentiometric analysis - as shown in Figure 1.

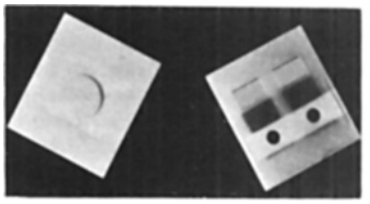

Figure 1 - Colorimetric and Potentiometric Slides

Figure 2 - Mounted Multilayered Material

Let us start by considering the Colorimetric slide technology. The Kodak technology makes use of a 16mm square chip of coated multilayer material which for structural strength is held in a plastic mount like a Kodachrome Transparency - as illustrated by Figure 2.

To Activate the coated reagents, the slide accepts 10 uL of serum or plasma. This starts a chain of reactions which results in the development of a colour. This colour is proportional in concentration to the analyte being measured. Now let us look at the structure of the coated layers.

The top layer we call a spreading layer. This layer acts as a metering device to deliver serum to the underlying reagent layers. It is coated from a solvent mixture which dries over the reagent layers as a very regular structure, with defined pore size. Different materials are coated in this requirements of the assays. The layer itself varies from 100 to 300 microns in thickness, depending on the assay to be performed. Average pore sizes range from 1.5 to 30 microns and the void volume from 60% to 90%. This structure allows a rapid and uniform spreading of sample over the underlayers. A spreading layer not only traps cells, crystals and other small particulate matter, but also retains large molecules such as protein. In essence, what passes through is a protein free filtrate.

Compounds that reflect light such as $TiO_2$ and $BSO_4$ may be incorporated into the spreading layer to mask coloured constituents in patient samples on the top side of the slide from the relectometer. They also provide a relectance background for the indicator layer which is the layer closest to the transparent support.

Figure 3 shows what happens when sample is dispensed on the spreading layer. After 10 uL of sample contacts the surface of the slide, capillary action draws sample into the porous layer. Sample is momentarily excluded by the gelatin-containing under-layers which require time to hydrate before they will accept serum components. It is this difference in times, spreading versus penetration of lower layers, that gives the benefit of the slide being less sensitive to sample volume fluctuation.

Moving from the spreading layer let us consider the underlying layers which serve to convert analyte in the sample to a quantifiable material while screening out interfering substances. One or more layers are coated beneath the spreading layer to perform a number of functions. The number of layers depends on the complexity of the chemical reactions.

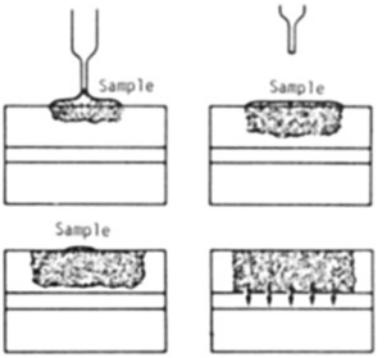


Figure 3 - Sample Metering

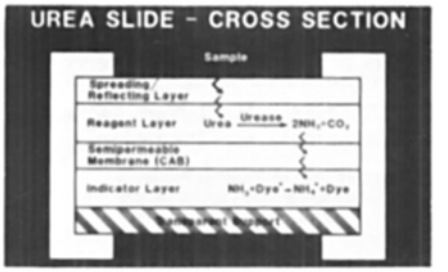


Figure 4 - Urea Slide

For example layers with special functions can be incorporated into the system to direct and specify the course of the chemical reactions. The UREA SLIDE as seen in Figure 4 has four layers coated onto a transparent support. Urease is coated in the top reagent layer and catalyses the hydrolysis of urea to ammonia and carbon dioxide. A very thin layer of CELLULOSE ACETATE BUTYRATE is coated beneath the urease layer, and acts as a gas permeable membrane. It allows the diffusion on ammonia, but minimises hydroxyl ion diffusion through to the indicator layer below. The ammonia diffuses into the indicator layer where it reacts with the leuco dye indicator.

Another example of specialized layers can be seen if we consider triglyceride as seen in Figure 5. The spreading layer is coated with a surfactant Triton 100 and the enzyme lipase. When serum is spotted onto this layer triglyceride is dissociated from lipoproteins and hydrolysed by lipase to glycerol. The glycerol passes through the scavenger layer to a glyceral detection layer where a series of enzymatic reactions take place which result in the formation of a coloured dye. The scavenger layer is so called because it is composed of Ascorbic Oxidase which removes ascorbic Acid, a known interferant in the oxidation reaction which produces the coloured indicator dye. These are only two examples of the many design options that are available to control the reaction sequence.

The final reaction is always a chromogenic reaction. Its purpose is to provide a quantifiable product that is directly proportional to the amount of analyte being assayed.

The amount of chromogen in the indicator layer is read using reflectance spectrophotometry - illustrated in Figure 6. The light passes through the indicator layer, is reflected from the bottom of a pigment-containing layer which is usually the spreading layer, and is returned through the side that receives the sample. This avoids the necessity of having to read the indicator layer through potentially

interfering materials that have been screened out by the overlying spreading layer. This is a major difference from familiar dipstick methods which are read through the same surface that receives the sample.

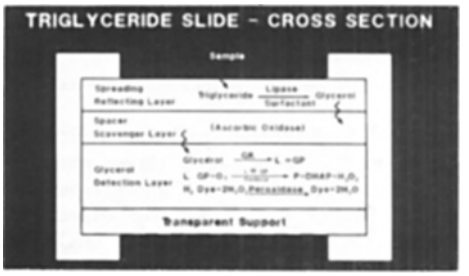


Figure 5 - Triglyceride Slide

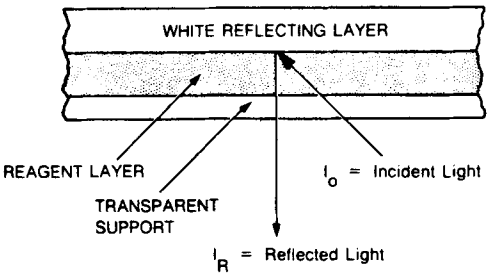


Figure 6 - Reflectance

Potentiometric analysis is also possible using the Dry layered format. This is achieved using two electrodes, one indicator electrode and a reference electrode are required for any potentiometric determination. Two small identical strips of the dry-operative flat Ion Selective Electrodes are mounted side by side as the indicator and reference electrodes - as shown in Figure 7. A reference fluid, containing the ions under test at known concentrations is applied as a 10 uL drop to the reference electrode.

10 uL of the test fluid is simultaneously applied to the indicator electrode. The two fluids form a liquid junction by a capillary flow through the small strip of ion-free paper, imbedded in a polystyrene cover sheet - illustrated in Figure 8. The polystyrene eliminates evaporation and also confines fluid contact to the drop holes. Electrical contact to the electrometer is made at the opposite end of the electrodes from the drop holes. The arrangement constitutes a concentration cell and has the property that any small drift on the test side is countered by any equal drift on the reference side. The potential difference between the two sides of the cell is translated into concentration units.

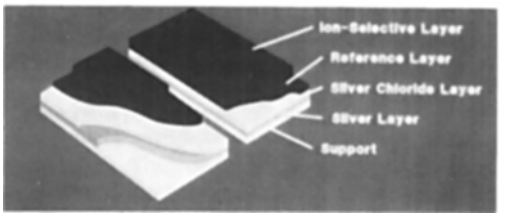


Figure 7 - Potentiometric Electrode

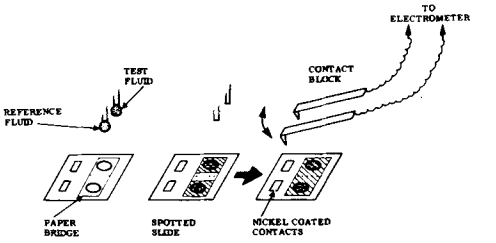


Figure 8 - Potentiometric Analysis

The choice of any one technological approach to serum analysis provides certain advantages, seen in Figure 9, and imposes constraints, as seen in Figure 10, when compared to any other technology. For example the thin film format and the use of reflection spectrophotometry, means the use of a single reaction pH. However, these constraints are more than balanced by, for example, the ability to mask background colour.

Kodak have delveloped a range of analysers specifically for handling the EKTACHEM slides. The EKTACHEM DT60 is the smallest analyser in the range, and it is suited to low volume testing such as that carried out in emergency laboratories, or in sattelite laboratories. It basically consists of two modules, one for colorimetric testing and one for potentiometric testing. During 1986, a third module will be available for the analysis of rate chemistries.

METHODOLOGICAL ADVANTAGES

Sequential Analytical Steps in One Cell
Selective Separations
Force Equilibria by Removing Product
Use of Non Water Soluble Reagents
Screen Proteins
Mask Background Colour
Homogenous Reaction Without Mixing

Figure 9 - Methodological Advantages

METHODOLOGICAL CONSTRAINTS

Short Pathlength
Single Reaction pH
Use of Undiluted Serum

Figure 10 - Methodological Constraints

The practical advantages of this technology are highlighted by the following examples. It has been possible to reduce calibration to once every three months, and Figure 11 shows the stability of Potassium and Urea over a 4 month period. Also the DT60 is a very easy to use system and Figure 12 shows the effect of multiple operators. Nineteen operators were trained in the use of the EKTACHEM DT60 and DTE Module. The training period which included an explanation of the system practical 'hands on' experience, took approximately 30-60 minutes. The operators were then asked to analyse quality control samples for Sodium Potassium, glucose and urea. The results obtained using two levels of control fluid for Potassium and urea, are shown here. Figure 11 and 12 have been reproduced by permission of Dr D. Burnett of St. Albans City Hospital, who has been involved in a long term evaluation of the system.

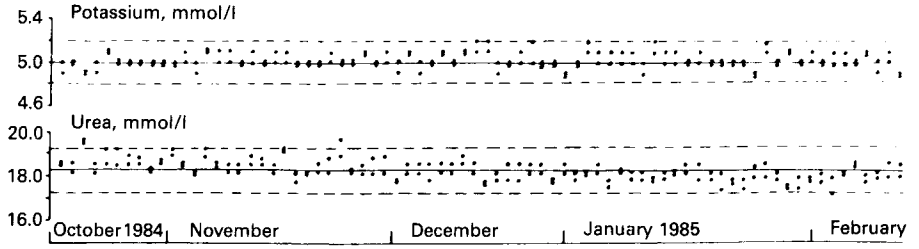


Figure 11 - Long Term Stability

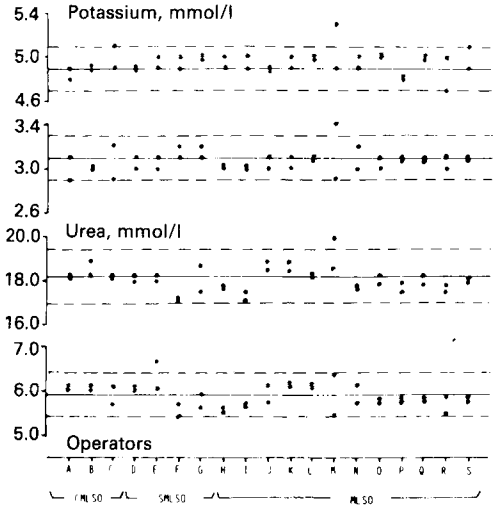


Figure 12 - Multiple Operators

| | AVAILABLE NOW | COMING SOON |
|---|---|---|
| DT-60 | Glucose | Creatinine |
| | Urea | Ammonia |
| | Cholesterol | Haemoglobin |
| | Triglycerides | |
| | Total Bilirubin | |
| | Total Protein | |
| | Amylase | |
| | Uric Acid | |
| DT-E | Sodium | |
| | Potassium | |
| | Chloride | |
| | Bicarbonate | |
| DT-R | | AST (ASAT) |
| | | ALT (ALAT) |
| | | ALKP (ALP) |
| | | CK (CK) |
| | | GGT (γGT) |
| | | LDH (LD) |

Figure 13 - Chemistry Menu

In summary the KODAK 'Ektachem' Clinical Chemistry programme is a growing one with an expanding range of available tests as shown in Figure 13.